

Gentamicin penetration and decay in the human aqueous

JEFFREY S. HILLMAN, S. I. JACOBS, A. J. GARNETT, AND M. B. KHESKANI

From St James's University Hospital, Leeds

SUMMARY The penetration of gentamicin into human aqueous was measured after intensive drops or subconjunctival injection of the antibiotic. The drop regimen proved ineffective in producing a therapeutically effective level. After a single 40 mg subconjunctival injection an effective concentration was achieved in 2 hours and maintained for 24 hours. The significance of these findings is discussed with respect to therapeutic implications.

Gentamicin is highly active against many of the bacteria that infect the human eye and is widely used in ophthalmology. It is particularly valuable in the immediate management of endophthalmitis where antimicrobial treatment must be instituted before bacteriological studies are completed. The antibiotic is usually administered locally as drops or by subconjunctival injection.

After the subconjunctival injection of 10 mg of gentamicin Furgiuele (1970) observed that therapeutically effective levels of the drug were attained in the aqueous of patients undergoing routine cataract extraction, and these findings were confirmed by Mathlone and Harden (1972), who used doses of 20, 40, and 80 mg. In contrast Ellerhorst *et al.* (1975) showed that 0.3% gentamicin drops failed to achieve a significant concentration in the aqueous of all of their patients except 1 even when the eye was inflamed, while MacIlwaine *et al.* (1974) found that the antibiotic failed to reach the aqueous in adequate amounts after a single intravenous dose of 1 mg/kg body weight. The clinical value of subconjunctivally injected gentamicin has been established (Goulstine and Marmion, 1971), but information is incomplete on the intraocular penetration of the drug in man and its subsequent decay in the aqueous.

The present study was undertaken to investigate the penetration of gentamicin into human aqueous from an intensive drop regimen compared with a single subconjunctival injection and to determine how long a therapeutically effective concentration would be maintained.

Material and methods

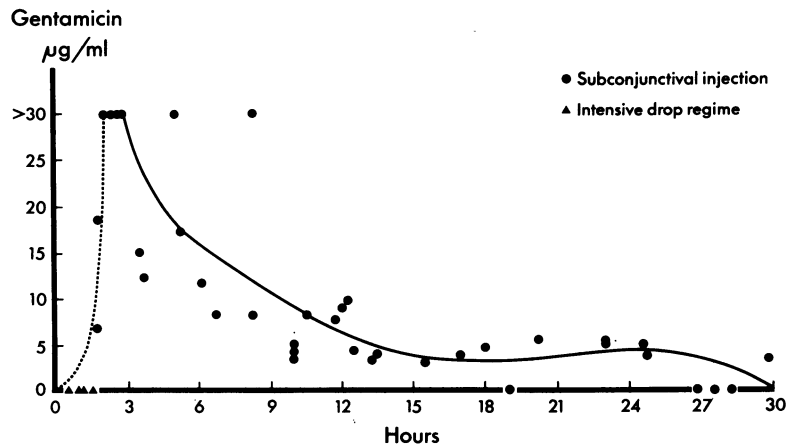
The study was conducted on 41 eyes in 40 patients who all presented for elective cataract extraction. The age range was between 56 and 92 years, and 16 were male and 24 female. The immediate preoperative preparation consisted of cocaine and adrenaline drops for 34 eyes or cyclopentolate drops alone for 7 eyes, given 30 minutes before surgery.

In 37 eyes the antibiotic drops routinely administered preoperatively were replaced by a single subconjunctival injection of 40 mg gentamicin contained in 1 ml, given at a range of times before surgery and made into the lower fornix after surface anaesthesia with amethocaine drops. Four eyes received instead intensive treatment with 0.3% gentamicin drops timed to finish at various intervals before operation. Approximately 2.5 mg of antibiotic was instilled during this treatment which followed a regimen of 1 drop per minute for 5 minutes followed by 1 drop every 5 minutes for 30 minutes and then by 1 drop every 15 minutes for 90 minutes.

Cataract extraction was performed under an operating microscope by the ab-externo technique. A limbal-based conjunctival flap was reflected and incision made at the corneoscleral junction down to Descemet's membrane. Haemostasis was obtained and the wound dried with a compressed sponge swab to remove any antibiotic which might have been released from beneath the conjunctiva. A small incision was then made through Descemet's membrane into the anterior chamber and about 0.2 ml of aqueous aspirated via a 30 gauge anterior chamber cannula into a 1 ml tuberculin syringe, which was sealed with a cap and sent for assay. Air was allowed to reform the anterior chamber, the section

Correspondence to J. S. Hillman, FRCS, Department of Ophthalmology, St James's University Hospital, Leeds LS9 7TF.

Fig. 1 Aqueous concentration of gentamicin after its administration by 2 hour intensive 0.3% drop regimen or by 40 mg subconjunctival injection



was completed, and cataract extraction was performed.

The microbiological assays were carried out in 10×10 inch (25×25 cm) plates by an agar diffusion method. 150 ml of antibiotic assay No. 1 agar (Mast Laboratories) seeded with a test strain of *Pseudomonas aeruginosa* donated by Nicholas Laboratories was poured into the plates, which had been levelled on an adjustable tripod. After drying, wells were cut in the plates with a No. 3 cork borer and 50 μ l of aqueous dispensed by an Eppendorf pipette into each of 3 wells in a random pattern. Standards containing 2.0, 5.0, 10.0, and 20.0 μ g gentamicin per ml were made up in distilled water and set up randomly in triplicate on every plate. After the application of standards and aqueous the plates were allowed to diffuse for 30 minutes at room temperature before overnight incubation at 37°C. The zones of bacterial inhibition were measured with a Luckham zone reader, and the mean diameters of the triplicate readings of the standards were plotted on semilogarithmic paper to give a straight line. The gentamicin content of the aqueous was calculated from this line extrapolated down to 1 μ g/ml and up to 30 μ g/ml.

Results

The results of the gentamicin bioassays are presented in Fig. 1, which relates the aqueous level of antibiotic to the time after completion of antibiotic delivery. The graph is a computer-fitted 4th degree polynomial curve obtained on a Wang 2200T computer using program f1-3-0 of Statistical General Program Library GLBR 22 A.

Drop therapy. The 4 aqueous samples taken between $\frac{1}{2}$ and $1\frac{1}{2}$ hours after completion of the 2-hour intensive drop regimen failed to show any detectable

gentamicin. This series was then discontinued, as there was no doubt that this method of delivery had failed to approach a therapeutic level in the aqueous.

Subconjunctival injection. After subconjunctival injection of 40 mg gentamicin the aqueous concentration rose to reach very high levels in excess of 30 μ g/ml after 2 hours. A high level was maintained for the first 12 hours and an adequate level for the second 12 hours after injection.

No observed differences between the gentamicin aqueous levels were attained which related to the age or sex of the patient. The postoperative clinical courses of the patients were unremarkable and there were no cases of intraocular infection.

Discussion

For an antibiotic to be effective a therapeutic concentration must be delivered to the infected tissues and maintained for an adequate period of time. The route of delivery has a great effect on the degree of penetration of a drug into the eye. When presented in drop form, absorption is thought to take place directly across the cornea. Contact time with the cornea is limited because of drainage down the lacrimonasal system, but it can be increased by increasing the frequency of drop instillation or by continuous irrigation. However, the results of the present investigation show that even after 2 hours' treatment with frequent 0.3% gentamicin drops little antibiotic reaches the aqueous in the noninflamed eye. If therapeutically effective aqueous levels of gentamicin are required, as in the management of endophthalmitis, topical drop delivery alone is inadequate.

The poor penetration obtained with topical drop therapy contrasts with the efficacy of subconjunctival injection in achieving high aqueous levels. The

figure shows that concentrations of more than 4 $\mu\text{g/ml}$ aqueous are attained within 1–2 hours of a single 40 mg subconjunctival injection and that by 2–3 hours very high levels of the antibiotic, in excess of 30 $\mu\text{g/ml}$, are present in the anterior chamber. The concentration of gentamicin falls over the first 12 hours to reach a level of about 4 $\mu\text{g/ml}$, which is maintained for the next 12 hours.

In the treatment of infective endophthalmitis a high concentration of antibiotic in the anterior chamber is essential, and a level of 4 $\mu\text{g/ml}$ is likely to be bactericidal for all the commonly encountered aerobic and facultatively anaerobic pathogenic bacteria except the streptococci (Garrod *et al.*, 1973). Obligate anaerobes are highly resistant to gentamicin, but streptococci, which are moderately resistant to gentamicin alone, frequently show a synergistic response to a combination of gentamicin and a penicillin (Watanakunakorn, 1971). A concentration of 4 μg gentamicin per ml in the aqueous represents the minimum inhibitory concentration of the drug for *Ps. aeruginosa*, which is refractory to many other antibiotics and a cause of grave endophthalmitis. The published values for the minimum inhibitory concentration of gentamicin for this organism vary widely because the results obtained are dependent on the magnesium content of the medium used in the test system (Garrod and Waterworth, 1969). As the magnesium ion concentration of the aqueous is approximately 80% of that found in plasma, it seems prudent to accept the high value of 4 μg gentamicin per ml as the minimum effective level in the aqueous for *Ps. aeruginosa* infections.

Although the present observations show the likely therapeutic value of a subconjunctival injection of 40 mg gentamicin, the design of the study did not allow the direct evaluation of the possibility of antibiotic accumulation in the aqueous after repeated injections, but *prima facie* this seems unlikely. Twenty-seven hours after a subconjunctival injection gentamicin was not detectable in the aqueous in 3 cases. In clinical practice a limiting factor for repeated subconjunctival injections is the clinical state of the conjunctiva, which may become

indurated. If this occurs, injections may be given into a deeper plane, as experiments in the monkey by Barza *et al.* (1978) have shown penetration with good posterior segment levels and smaller anterior segment levels on retrobulbar injection of gentamicin.

A theoretical risk from repeated subconjunctival injections of gentamicin in patients with impaired renal function is the possibility of systemic accumulation of the drug with risk of toxicity. It is most unlikely that a single 40 mg injection of gentamicin would prove hazardous in a patient of average build even with poor renal capacity, but, if injections are frequent or renal function impaired, the serum level of the antibiotic should be monitored and therapy modified accordingly.

We thank Mr F. T. de Dombal for statistical advice and the Department of Medical Illustration at St James's University Hospital for the preparation of the Figure.

References

- Barza, M., Kane, A., and Baum, J. (1978). Intraocular penetration of gentamicin after subconjunctival and retrobulbar injection. *American Journal of Ophthalmology*, **85**, 541–547.
- Ellerhorst, B., Golden, B., and Jarudi, N. (1975). Ocular penetration of topically applied gentamicin. *Archives of Ophthalmology*, **93**, 371–378.
- Furguele, F. P. (1970). Penetration of gentamicin into the aqueous humour of human eyes. *American Journal of Ophthalmology*, **69**, 481–483.
- Garrod, L. P., and Waterworth, P. M. (1969). Effect of medium composition on the apparent sensitivity of *Pseudomonas aeruginosa* to gentamicin. *Journal of Clinical Pathology*, **22**, 534–538.
- Garrod, L. P., Lambert, H. P., and O'Grady, F. (1973). *Antibiotic and Chemotherapy*, 4th ed., pp. 124–125. Churchill Livingstone: Edinburgh and London.
- Goulstine, D. B., and Marmion, V. J. (1971). Subconjunctival gentamicin. *British Journal of Ophthalmology*, **55**, 478–480.
- MacIlwaine, W. A., Sande, M. A., and Mandell, G. L. (1974). Penetration of antistaphylococcal antibiotics into the human eye. *American Journal of Ophthalmology*, **77**, 589–592.
- Mathlone, M. B. R., and Harden, A. (1972). Penetration and systemic absorption of gentamicin after subconjunctival injection. *British Journal of Ophthalmology*, **56**, 609–612.
- Watanakunakorn, C. (1971). Penicillin combined with gentamicin or streptomycin: synergism against enterococci. *Journal of Infectious Diseases*, **124**, 581–586.